of the subject from the point of view of physiological plant anatomy. That point of view is for the present, however, somewhat under a cloud in this country because it does not appeal to the morphologist and the evolutionist on the one hand or to the cultivator of the disembodied plant physiology at present in vogue in these United States, on the other. When the physiologist among us again begins to recognize the importance of plant structures, he will possibly find a work conceived in this manner useful.

E. C. Jeffrey

America's Greatest Problem: the Negro. By R. W. Shufeldt, M.D., major, medical corps, United States Army, member Association of American Anatomists, fellow of the American Ornithologists' Union, etc. Philadelphia, 1915. Roy. 8vo, pp. 377, with fifty-two illustrations.

Unfortunately this volume has been heralded as "a wonderfully startling book . . . certain to instantly arouse a vigorous nation-wide discussion," and—by implication—as "an authoritative . . . guide to the solution of this menace of the deterioration of the Caucasian race in America." Nevertheless (these hyperboles being credited to the mercantile enthusiasm of the publishers, whose part has been done quickly and well), a notice of it was undertaken by the present writer partly because of his interest in the Negro, and partly because he took for granted that the author, a well-known ornithologist and comparative anatomist, would materially increase our knowledge of the facts involved, facilitate our comprehension of the nature and causes of the existing undesirable relations between the races, and offer something novel as "a remedy whereby the peril may be safely passed."

These expectatons have not been met. On the contrary, while the author's earnestness is evident, a careful and unprejudiced examination of the volume leads the reviewer to wish that the time and energy expended upon it had been devoted to the strictly scientific work which the author had in hand (p. vii); that might, at least, have been free from the needlessly frequent references to topics connected with *psychopathia sexualis* which characterize this and some of his other publications.

Burt G. Wilder

SPECIAL ARTICLES

ZYGOSPORES AND RHIZOPUS FOR CLASS USE

Rhizopus nigricans—the common bread mold—is the form most frequently used in the microscopic study of fungi in elementary classes in botany. Its production of both sexual and non-sexual spores, added to the ease with which it may be obtained and grown without refined laboratory facilities, makes it an ideal form for class study. The zygospores, though not difficult to find, have been overlooked by most teachers and many requests have been made of the writer for information in regard to methods of obtaining them. It has seemed desirable therefore to publish a short note on the subject.

Rhizopus is commonly found in nature on decaying fruits and vegetables as well as upon bread which has been kept in a moist atmosphere. The air is so full of its spores that almost any substratum rich in carbohydrates, if kept under proper moisture conditions, will produce a spontaneous growth of the fungus. The essential precaution is to insure a moist atmosphere and at the same time to prevent the substratum itself from becoming so moist as to stimulate the growth of bacteria. A simple method is to line a tumbler with moistened filter paper or even newspaper and to place a piece of bread on some non-absorptive object inside that will keep it from contact with the moist paper on the sides and bottom. The bread should be moist but not wet—the consistency of fresh bread is ideal—and the container should be kept closed. A bell jar lined with moist filter paper covering a dish with water or moist paper on the bottom, also makes a good moist chamber. Within a week, if the air has been kept moist, a good growth of the mold will result. Green molds will often be present as well, but the *Rhizopus* is so rapid in growth that contamination with other forms will not generally be seriously troublesome. Zygospores will sometimes be

found in the lower and moister parts of the culture below the sporangial growth but need never be expected in the upper and dryer regions. They frequently form in pure masses free from sporangia in the folds of crumpled paper directly below the substratum. The species, however, is diecious and it may frequently happen that the spores of only one of the two sexes, which together are necessary for zygospore production, have fallen upon the substratum. The chances of finding zygospores in the lower parts of a spontaneous culture will be increased if bits of the fungus from different sources in the field are laid on the bread when the culture is started. The two sexes are often found growing together in nature although the moisture conditions may not be such as to cause the appearance of zygospores. Thus, out of seven mixed transfers made this last month from as many individual decayed squashes in the field, three showed the presence of the two sexual races by production of zygospores, and of the remainder one was male and three were female. If even a small mass of zygospores is found, their production can be increased by laying on a fresh culture of bread, a bit of such zygosporic material freed as much as possible from sporangial spores. When an abundant production of zygospores has been once secured, the bread with the fungus growing on it can be dried and kept six months or more to be used later as "spawn" if broken up and laid on fresh bread when a zygosporic culture is again desired. Cultures have been kept over a year in this way but the spores are relatively short lived and fresh "spawn" should be prepared at more frequent intervals.

Zygospores of *Sporodinia* also may be readily obtained from sowings of the spores on bread in moist chambers. This mold is hermaphroditic. If a large collection of different fleshy fungi are left for a few days under a bell jar, a sporangial growth of *Sporodinia* will usually be found appearing on some of the decaying fungi.

In studying the habit of growth of the bread mold, pieces of paper on which the mold has spread will be found convenient or masses of the mold-infected bread (about half the size of an English walnut) which is beginning to show sporangia may be left for 24 to 48 hours in Petri dishes. If the Petri dishes are kept in too moist an atmosphere, stolons with but scanty sporongia may result, if kept too dry no stolons will be produced. If the Petri dishes be wrapped in paper, the proper conditions are generally secured. The presence of the columella within the unopened sporangium may be shown by drawing a solution of KOH under the cover.

To observe the swelling and germination of the sporangial spores, they may be sown in suitable fluid media or on nutrient agar. The filtrate obtained after boiling a couple of prunes for five or ten minutes in 100 c.c. of water makes a convenient fluid for the purpose without the necessity of further sterilization. Only enough spores should be added to make the fluid slightly clouded or to give a sufficient number of germinations in a drop taken for observation. At room temperature the formation of germ tubes may be expected in five or six hours. The process may be hastened or delayed by keeping the spores at higher or lower temperatures.

The methods suggested in the preceding paragraphs have been purposely such as can be adopted by any teacher without the facilities for sterilization. It will not be necessary to give detailed directions to those familiar with cultural methods. It may be said, however, that *Rhizopus* and especially its zygospores develop best upon nutrients rich in carbohydrates. If agar is used, 4 per cent. to 8 per cent. dextrose will be found a desirable ingredient of any formula. Bread is an ideal substratum but ordinary sterilization renders it pasty and unfit for use. A short sterilization with steam, but not under pressure, continued for less than five minutes will probably be found satisfactory.

The two sexes may be isolated from a culture, producing zygospores by making transfers from individual sporangia to well-separated points in the outer margin of a large dish of some suitable substratum. Zygospores will appear between the growths of oppo-

site sexes. Since one of the sexes may predominate in sporangial growth, the writer has found it a surer method to pick out with fine needles young zygospores free from sporangial spores and to plant them in Petri dishes on nutrient agar. One or both suspensors are likely to grow into mycelia which can be tested out as suggested above.

Inoculation of many sporangial spores causes a dense growth of small sporangia and a reduction of the mycelial growth at the point of inoculation. It is therefore advisable to inoculate only a small number of spores when desiring zygospore production or better yet, to make transfers of the mycelia from fresh tubes of the fungus before they have produced sporangia. In either way the opposite sexes may be sown together or slightly separated so as to cause a somewhat indefinite mass of zygospores where the opposing growths meet. If the nutrient requirements are satisfied and the atmosphere is kept saturated, zygospores may be thus obtained in abundance and nearly free from sporangia.

To teachers on my regular exchange list I am planning to send out dried male and female spore material of *Rhizopus* for use with their classes, together with reprints of the present article. I should also be glad to supply any other teachers with this material who may request it. Cultures should be started from this dried material within a month's time. The male and female cultures may be kept running by transfers to fresh nutrient about every three or four months.

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Notes on the Factors Involved in the Germicidal Effect of Freezing and Low Temperatures

Many interpretations and conclusions on the germicidal activity of low temperatures and freezing have been given by earlier investigators. Cold was formerly considered a powerful disinfecting agent, but now there is a tendency to emphasize other factors than cold itself as potent. In fact we know that cold may act as a preserver of germ life, as the high bacterial content of frozen food stuffs after weeks and months of refrigeration indicates. Ice, on the other hand, tends to purify itself upon storage.

There are numerous variables which may have an important bearing upon the experiments. A partial list of these includes: (1) The species or strain of bacteria used, (2) the history and cultural manipulation of the organism prior to freezing, (3) the physical and chemical composition of the medium in which the organism is frozen, (4) the temperature of the frozen mixture, (5) the duration of the freezing, (6) the abruptness of temperature changes, (7) the cultivation of the organism subsequent to freezing. This list includes those factors which we took special pains to control.

The bacteria may be killed by the mere fact of low temperature interfering with metabolism; by freezing of the cell contents and rupture of the membrane by internal pressure; by external pressure or grinding developed during crystallization, or by expansion of the frozen medium within the receptacle; or by more or less prolonged suspension of metabolic activities, leading to slow death from old age or starvation.

We shall not take the space to give more than a summary of our preliminary results.

I. The comparative germicidal potency of freezing on different species and strains of bacteria.

B. coli and *B. subtilis* (twenty-four hour old cultures, the latter presumably practically spore-free), showed the former species to be much more susceptible to freezing. Ninety-nine per cent. and over of the *B. coli* succumbed to freezing in tap water in three hours, while with *B. subtilis* the reduction was not at all uniform, but seldom exceeded eighty per cent. Three strains of *B. coli* tested showed no appreciable variability in relation to the disinfecting influence of cold and freezing.[1]

[1] The remainder of our experiments were performed with *B. coli*.